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PHYTOCHEMICAL PHARMACOGNOSTICAL ANTIMICROBIAL AND CYTOTOXICITY STUDIES ON *CANTHIUM COROMANDELICUM* FRUIT

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ABSTRACT: The medicinal plants are the key source in the life of human beings. The present study was aimed to evaluate the preliminary phytochemistry, pharmacognostical and antimicrobial activity of *Canthium coromandelicum* fruit. The fruit showed the presence of secondary metabolites like flavonoids, steroids, tannins, phenol, sterols, cardiac glycoside and saponin glycoside in various extracts. The pharmacognostical analysis of *C. coromandelicum* was useful in the identification of authentic samples and recognizing adulterants. The various extracts of *C. coromandelicum* fruit has shown antimicrobial activity against eight bacterial strains including Gram negative, Gram-positive bacteria, and three fungal strains using agar well diffusion method. The methanolic extract showed the broad spectrum antimicrobial activity compared to petroleum ether and chloroform extracts. Chloroform extract showed high cytotoxicity value with low IC_{50} values. The antimicrobial studies proved that *Canthium coromandelicum* fruit has antimicrobial activity against the selected pathogens. The Brine Shrimp Lethality Assay is an excellent predictive tool for the toxic potential of plant extracts in humans.

INTRODUCTION: Plants are the richest source of medicinal drugs. India is one of the richest Bio Source nations in the world. In India, infectious diseases are still a challenging health problem. The children are majorly affected by the infections such as morbidity and mortality. Infections due to a variety of bacterial etiologic agents, such as pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Shigella* sp., *Enterobacter* sp. are most common [1].

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In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide [2]. It is aroused due to heterogeneous and repetitive use of antimicrobial drugs.

The harmful microorganisms can be controlled with drugs and these results in creating an alarm for the clinical situation against the infections. Brine shrimp lethality assay have been used as a bench top bioassay for the discovery and purification of bioactive natural products. This assay is highly useful tool for preliminary assessment of toxicity, for the detection of fungal toxins, plant extract toxicity, and cyanobacteria toxins [3]. Brine shrimp tests are performed to know the safety of the plant extracts and also illustrate trends of their biological

activities up to an extent. *Canthium coromandelicum* belongs to the family Rubiaceae is a bushy thorny herb. Canthium is a genus of about 230 species of shrubs. It occurs in coastal regions and dry plains of India and also in Srilanka. Its leaves are simple, small and obovate, opposite with interpetiolar stipules and axillary spines. Its leaves and roots are invaluable for their medicinal importance. Macerated paste of leaf is externally applied twice a day to treat scabies and the ring worm infection [4].

Canthium as herbal medicine is used for the treatment of diabetes among major tribal groups in South Tamil Nadu. The various species of Canthium has been reported for their antioxidant, wound healing activity, antitumor activity, antimicrobial, anti-diabetic, diuretic, anti-inflammatory, anti-nociceptive, and antipyretic [5]. Hence the present study was intended to evaluate the phytochemical screening, pharmacognostical and antimicrobial activity of *Canthium coromandelicum* fruit was tested against human pathogens.

MATERIALS AND METHODS:

Collection of Plant Materials: The fruits of *C. coromandelicum* were collected from foot Hills of Western Ghats, Sivanthipuram during the month of August. The plant was identified by Dr. M. Johnson, Assistant Professor of Botany, St. Xavier's College (Autonomous), Palayamkottai and the voucher specimen (XCH 26878) was deposited in St. Xavier's College Herbarium, Palayamkottai for future reference.

Preparation of Extract: About 60g of air-dried and coarsely powdered fruit of *C. coromandelicum* were extracted using petroleum ether, chloroform and methanol through Soxhlet method successively. The extraction was carried out about 72 hours, concentrated using vacuum evaporator and take over for further analysis.

Preliminary Phytochemical Screening: Preliminary phytochemical screening was carried out by the standard procedure [6]. The extract was tested for both primary and secondary metabolites such as steroids, alkaloids, triterpene, sugar, phenolic groups, flavonoids, catachin, saponin, tannin, anthraquinone, amino acid and reducing sugar.

Pharmacognostic Studies:

Fluorescence Analysis: For fluorescence analysis, air-dried powdered plant material and the extract of the powdered plant material in solvent such as petroleum ether, chloroform and methanol were examined under Visible light and Ultra Violet light (UV-365nm) [7]. The powder was treated with various chemical reagents such as 1N sodium hydroxide, 1N hydrochloric acid, sulphuric acid, nitric acid, acetic acid, acetone, ethanol, water, and ammonia. The changes in fluorescent behaviour were recorded.

Physico-Chemical Character:

Quantitative Determination: The percentage loss of weight on drying, total ash, water soluble ash, acid insoluble ash, and sulphated ash residue on ignition were obtained by employing standard methods of analysis [8].

Qualitative Determination of Ash Mineral Constituents: Qualitative determination of ash for mineral constituents was carried out by the standard procedure as follows:

- Take a trace of ash (about $1/20^{th}$) in a test tube, 2-3 drops of dilute nitric acid and 3-4mL of distilled water, mix them and add a few drops of silver nitrate. A white precipitate is obtained. Presence of chloride.
- Take 1mL of ash solution in a test tube, add 2-3 drops of HCl, warm and add 10 drops of Barium chloride. A white precipitate is obtained. Presence of sulphate.
- Take 1mL of solution and add 3-4 drops of nitric acid boil them and add 4-6 drops of Ammonium molybdate. Add 5 drops of ammonium hydroxide and keep the tube in water bath at 60 °C. Yellow precipitate soluble in sodium hydroxide. Presence of phosphate.
- Take 1mL of solution and add 3-4 drops of potassium thiocynaide. A reddish brown precipitate is obtained. Presence of iron.
- Take 1mL of solution and add 3-4 drops of ammonium oxalate warm and add 3-4 drops of Ammonium hydroxide. A white precipitate soluble in dilute sulphuric acid. Presence of calcium.
- Take 1mL of solution and add few drops of ethyl alcohol and few crystals of tartaric acid. Close the mouth of the test tube with thumb and

shake thoroughly for 1-2 minutes. A white crystalline precipitate is obtained. Presence of potassium.

- Take 1mL of solution and solid Ammonium chloride and add excess of Ammonium hydroxide along the sides of the tube with glass rod. A white precipitate is obtained. Presence of Magnesium.
- Take 1mL of solution and neutralize with few drops of Ammonium hydroxide. Add about 1mL of Sodium bicarbonate or Potassium nitrate. Mix and add bromine in excess. An apple green colour is obtained. Presence of cobalt.
- Take 1mL of solution and few drops of acetic acid. Mix and add 5-6 drops of potassium ferrocynaide. A chocolate brown precipitate is obtained. Presence of copper.

Antimicrobial Activity: The antimicrobial activity was carried out by the Kirby-Bauer method.

RESULTS: The *Canthium coromandelicum* were subjected to preliminary phytochemical screening, pharmacognostical analysis and antimicrobial activity which were found to be very promising. The results of phytochemical screening of primary and secondary metabolites are tabulated in **Table 1** and **2**. The primary metabolites such as reducing sugar, sugar, gum are present in petroleum ether, chloroform as well as methanol extracts. Carbohydrate is present only in methanol extracts. Secondary metabolites such as phenol, steroid were present in methanol extract and cardio glycoside, sterol are present in petroleum ether and methanol extracts. Flavonoids, tannins are present in chloroform and methanol extracts. Saponins glycoside present in all the three extracts of *C. coromandelicum* fruit.

TABLE 1: PHYTOCHEMICAL SCREENING TEST FOR VARIOUS EXTRACTS OF *C. COROMANDELICUM* FRUIT

Primary Metabolites	Petroleum Ether	Chloroform	Methanol
Reducing sugar	+	+	+
Protein	-	-	-
Sugar	+	+	+
Amino acid	-	-	-
Fats and fixed oil	-	-	-
Gums	+	+	+
Mucilage	-	-	-
Carbohydrate	-	-	+

TABLE 2: PHYTOCHEMICAL SCREENING TEST FOR VARIOUS EXTRACTS OF *C. COROMANDELICUM* FRUIT

Secondary Metabolites	Petroleum Ether	Chloroform	Methanol
Phenol	-	-	+
Alkaloid	-	-	-
Cardio glycoside	+	-	+
Anthraquinone glycoside	-	-	-
Saponin glycoside	+	+	+
Coumarin glycoside	-	-	-
Flavonoids	-	+	+
Catachin	-	-	-
Sterols	+	-	+
Steroids	-	-	+
Terpenoids	-	-	-
Tannin	-	+	+

Fluorescence Analysis: For Fluorescence studies the powdered drug was studied and observations are recorded and tabulated in **Table 3**. These microscopical observations help to resolve botanical identity of the chosen plant specimens. The powdered drug in various chemical reagents showed characteristic fluorescence at 365nm of Ultra-Violet light which can play significant role as diagnostic tools in quality control of crude drugs.

TABLE 3: FLUORESCENCE ANALYSIS OF *C. COROMANDELICUM* FRUIT

Powdered drug	Visible light	UV (365nm)
Powder drug as such	Pale green	Green
Powder + 1N NaOH	Dark brown	Dark green
Powder + 1N HCl	Reddish brown	Intense green
Powder + H_2SO_4	Orange	Dark green
Powder + HNO_3	Pale yellow	Light green
Powder + CH_3COOH	Pale yellow	Light green
Powder + Acetone	Pale yellow	Light green
Powder + Ethanol	Pale yellow	Light green
Powder + H_2O	Pale yellow	Light green
Powder + NH_3	Light brown	Green

Physico - Chemical Studies: The Physico-chemical analysis and physical parameters of *C. Coromandelicum* fruit are tabulated in **Table 4** and **5**.

TABLE 4: PHYSICO CHEMICAL ANALYSIS OF *C. COROMANDELICUM* FRUIT

S. no.	Ash test	Percentage (w/w) of ash
1	Total ash	2.9592
2	Water soluble ash	1.1418
3	Acid insoluble ash	1.3497
4	Sulphated ash	5.2781

The physical parameter such as colour, consistency, solubility, extractive value and moisture content are also shown below.

TABLE 5: PHYSICAL PARAMETERS OF *C. COROMANDELICUM* FRUIT

S. No.	Parameters	Extracts		
		Pet. ether	Chloroform	Methanol
1.	Colour	Pale green	Dark green	Brownish red
2.	Consistency	Non-sticky	Sticky	Sticky
3.	Solubility in water	Insoluble	Insoluble	Soluble
4.	Extractive value(% w/w)	0.8815	0.990	9.8273
5.	Moisture		71.821	

Determination of Minerals: A number of inorganic elements are present in ash of chosen plant material. The existence of such inorganic elements in plants can be detected qualitatively. The qualitative determinations of inorganic minerals in ash are tabulated in **Table 6**.

TABLE 6: QUALITATIVE ANALYSIS OF ASH MINERALS OF *C. COROMANDELICUM* FRUIT

S. no	Mineral	Result
1	Chlorides	-
2	Sulphate	+
3	Phosphate	+
4	Iron	-
5	Calcium	-
6	Potassium	+
7	Magnesium	-
8	Cobalt	-
9	Copper	-

(+) - Presence, (-) - Absence

Antimicrobial Activity: The present study, *in-vitro* antimicrobial activity of *C. coromandelicum* fruit extract against Gram-positive and Gram negative bacterial strains and 3 fungal strains showed a broad spectrum of antimicrobial activity. The antimicrobial activities of plant extract are compared with standard antibiotics such as Amikacin and Fluconazole which were used as controls. The extract was treated against eight bacterial strains such as *Esherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus*, *Streptococcus*, *Bacillus cereus* and *Bacillus subtilis.* The extract was treated with three fungal strains such as *Aspergillus fl*avus, *Aspergillus oryzae* and *Actinomyces*. The results are tabulated in **Table 7**, **8** and **9** respectively. The zone of inhibition against pathogens was shown in **Fig. 1**, **2** and **3**.

TABLE 7: ANTIMICROBIAL ACTIVITY OF PETROLEUM ETHER EXTRACTS OF *C. COROMANDELICUM* FRUIT

S. no	Pathogens	Zone of inhibition (mm)				Control
		25µL	50µL	75µL	100µL	(Amikacin)
1.	*Escherichia coli*	-	-	-	-	22
2.	*Klebsiella pneumoniae*	8	9	11	-	23
3.	*Pseudomonas aeruginosa*	-	-	-	-	24
4.	*Staphylococus aureus*	-	-	10	-	23
5.	*Enterococcus*	-	-	16	25	25
6.	*Streptococcus*	-	10	12	14	21
7.	*Bacillus cereus*	-	-	-	-	28
8.	*Bacillus subtilis*	8	9	13	9	25
		Fungi				Fluconazole
9.	*Aspergillusflavus*	-	-	22	30	-
10.	*Aspergillusoryzae*	-	17	20	27	-
11.	*Actinomyces*	-	12	22	29	-

TABLE 8: ANTIMICROBIAL ACTIVITY OF CHLOROFORM EXTRACTS OF *C. COROMANDELICUM* FRUIT

S. no	Pathogens	Zone of inhibition (mm)				Control
		25µL	50µL	75µL	100µL	(Amikacin)
1.	*Escherichia coli*	-	-	-	-	19
2.	*Klebsiella pneumoniae*	-	9	14	-	23
3.	*Pseudomonas aeruginosa*	-	-	-	-	23
4.	*Staphylococus aureus*	-	-	-	-	23
5.	*Enterococcus*	-	12	18	25	24
6.	*Streptococcus*	-	-	14	-	19
7.	*Bacillus cereus*	-	8	12	-	26

8.	*Bacillus subtilis*	-	-	-	-	22
		Fungi				Fluconazole
9.	*Aspergillus flavus*	8	15	22	27	-
10.	*Aspergillus oryzae*	-	16	20	28	-
11.	*Actinomyces*	9	10	25	32	-

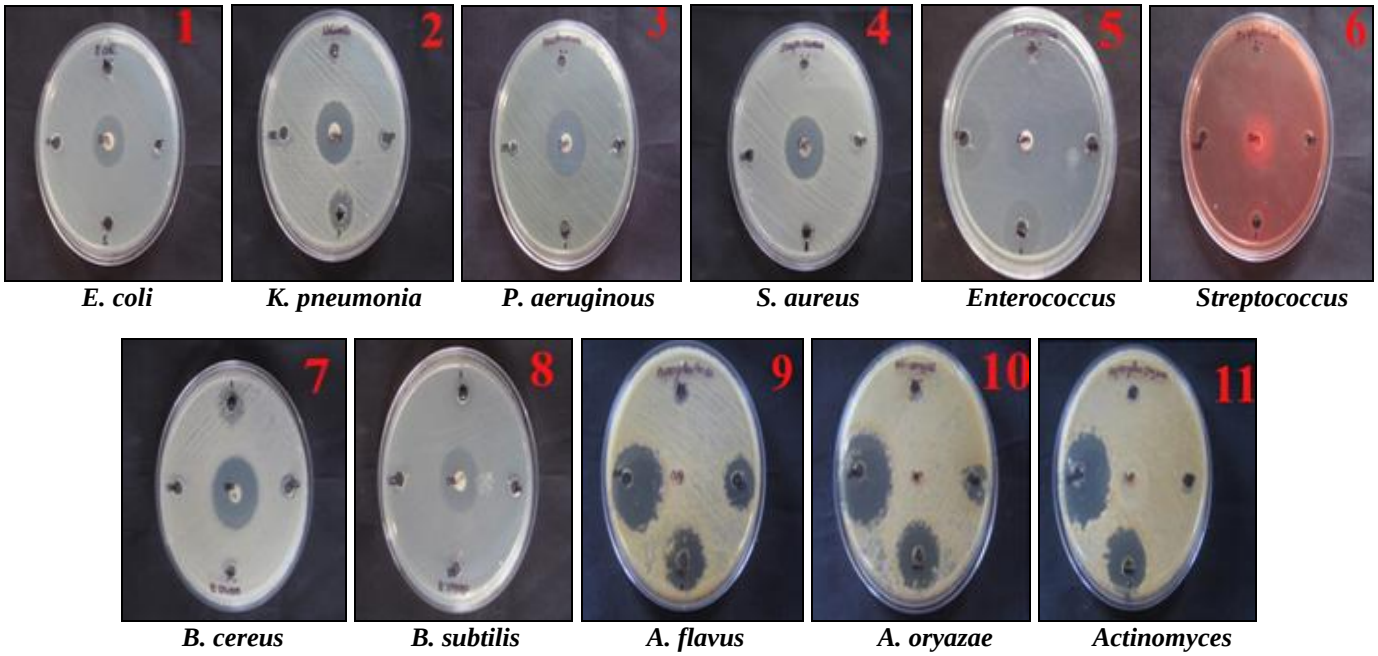

FIG. 1: ANTIMICROBIAL ACTIVITY OF PET ETHER EXTRACT OF *C. COROMANDELICUM* FRUIT

TABLE 9: ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACTS OF *C. COROMANDELICUM* FRUIT

S. no	Pathogens	Zone of inhibition (mm)				Control
		25µL	50µL	75µL	100µL	(Amikacin)
1.	*Escherichia coli*	10	10	09	23	25
2.	*Klebsiella pneumoniae*	-	-	10	14	21
3.	*Pseudomonas aeruginosa*	-	-	09	11	20
4.	*Staphylococus aureus*	-	08	11	17	27
5.	*Enterococcus*	-	-	10	16	15
6.	*Streptococcus*	-	-	-	-	23
7.	*Bacillus cereus*	-	-	-	10	26
8.	*Bacillus subtilis*	08	08	10	11	21
		Fungi				Fluconazole
9.	*Aspergillusflavus*	-	10	12	22	-
10.	*Aspergillusoryzae*	-	-	13	26	-
11.	*Actinomyces*	-	10	12	22	-

Cytotoxic Assay: Cytotoxic activity of pet ether, chloroform and methanolic fruit extracts of *C. Coromandelicum* using brine shrimp lethality assay, the results are tabulated in **Table 10**.

TABLE 10: CYTOTOXICITY OF VARIOUS EXTRACTS OF *CANTHIUM COROMANDELICUM* FRUIT

S. no.	Extract	LC_{50} (ppm)	95% confidence limit		LC_{90}	χ^2
			Lower	Upper	(ppm)	
1.	Petroleum ether	108.52	83.29	190.74	249.36	0.769
2.	Chloroform	32.81	337.54	67.901	122.92	6.873
3.	Methanol	62.80241	62.80241	62.80241	181.76	4.653

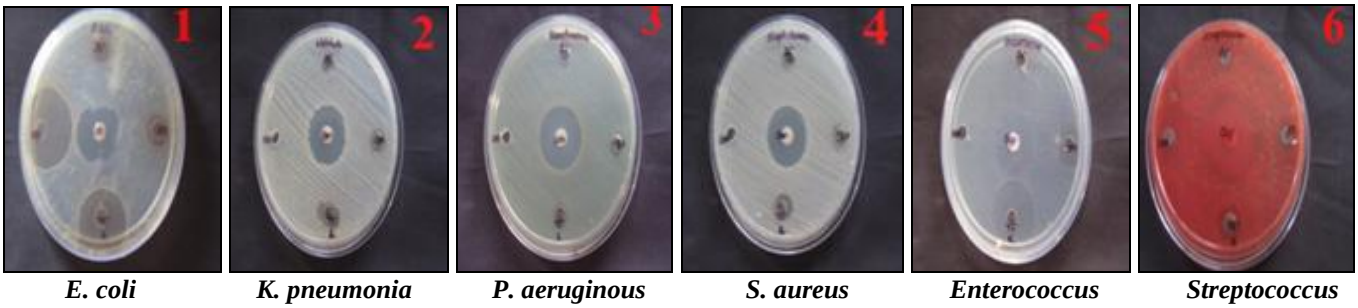

FIG. 2: ANTIMICROBIAL ACTIVITY OF CHLOROFORM EXTRACTS OF *C. COROMANDELICUM* FRUIT

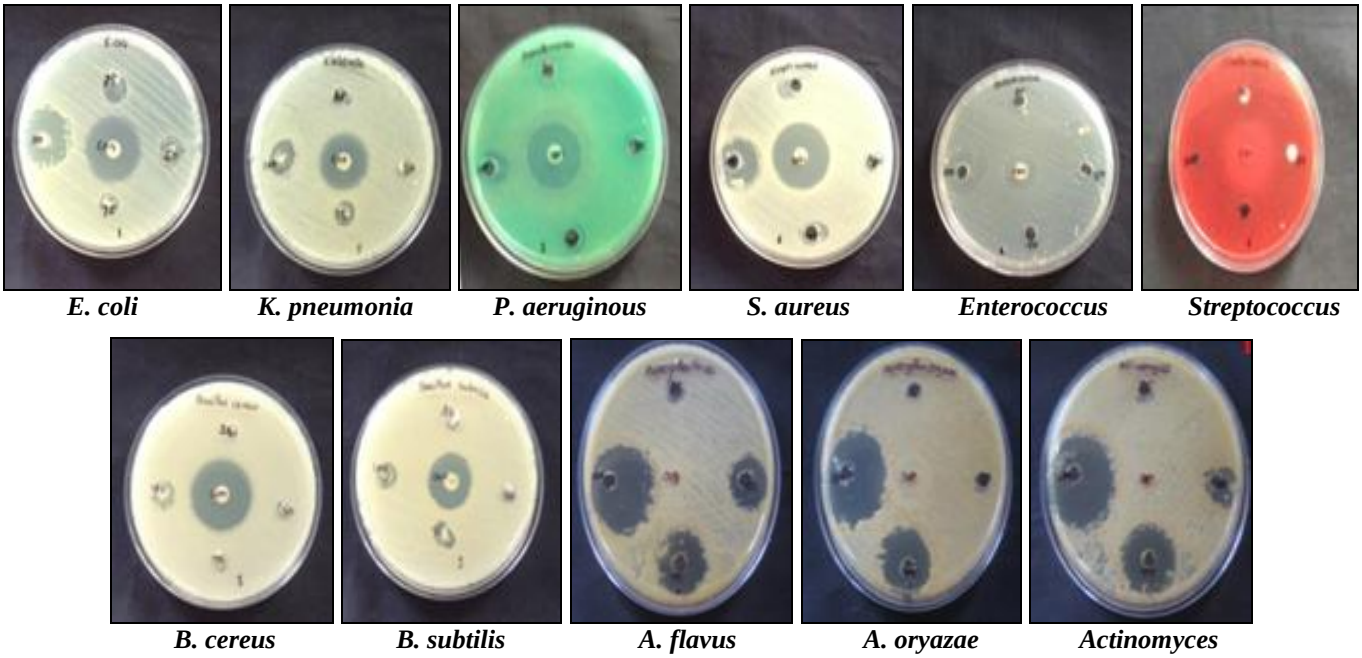

FIG. 3: ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT OF *C. COROMANDELICUM* FRUIT

DISCUSSION: The preliminary phytochemical screening may be useful in the detection of metabolites and highly in the drug discovery and development. The secondary metabolites contribute significantly towards the biological activities of medicinal plants such as antimicrobial, hypoglycaemic, anti-diabetic, antioxidant, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, anti-leprosy activities *etc* [9]. Secondary metabolites such as flavonoids, tannins, steroids, sterol, cardio glycoside and saponins glycoside were present in *C. coromandelicum* fruit. The methanolic extracts of *C. Coromandelicum* fruit contain maximum metabolites comparative to petroleum ether and chloroform extracts. Total ash values and extractive values were useful in identification and authentication of the plant material. Extractive values were useful to evaluate the chemical constituents of crude drugs. The total ash values were higher than the water soluble ash, acid insoluble ash and sulphated ash values in fruit. The total ash usually consists of phosphates, sulphates and potassium. A high ash value shows the presence of contamination, substitution,

adulteration, or carelessness in preparing the crude drug for marketing [10]. Thus all the physicochemical parameter of plant drugs reported here for the first time, is an important for detecting adulteration or improper handling of drugs which would be useful in standardization of herbal drugs. The extractive value of *C. Coromandelicum* fruit is maximum for the polar methanolic extract. Fluorescence analysis is one of the pharmacognostical procedures useful in the identification of authentic samples and recognizing adulterants.

In the present study, the fluorescence analysis was evaluated for fruit of *C. coromandelicum* in their powdered form or in different solvents and reagents. The fluorescence properties of the plant parts have been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs [11]. Several works on this aspect were reported in various plants to standardization of herbal drugs in quality control [12].

Thus the present study with detailed fluorescence analysis of the coarsely powdered drugs of *C. coromandelicum* fruit under UV and visible light reported here for the first time would be very useful aid to confirm authenticity and to check adulterants in crude form. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [13]. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [14].

In the present investigation, different extracts of *C. coromandelicum* was evaluated for their antimicrobial activity against certain Gram positive, gram negative bacteria and fungi. The implication of the broad spectrum action of some of these extracts was that they can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principle can be isolated [15]. In the present study, *Enterococcus* showed more activity in petroleum ether and chloroform extracts of *C. coromandelicum* fruit. Methanol extract showed maximum activity against *E. coli*, *Staphylococcus aureus*. 100µL of pet. ether, chloroform and methanol extracts of *C. coromandelicum* fruit showed better activity against *Aspergillus flavus, Aspergillus oryzae*, *Actinomyce*. Secondary metabolites which are of great importance in the field of drug research. These classes' alkaloids, Saponin, tannins, flavonoids are known to have activity against pathogens and therefore aid the antimicrobial activities of medicinal plants [16]. From the present study, it was found that crude extract express good biological activity which indicates that the substance has powerful biological effect. Therefore, medicinal plants are finding their way into pharmaceuticals, nutraceuticals and food supplements.

Cancer continues to represent the largest cause of mortality in the word and claims over 6 million lives each year. An extremely promising strategy for cancer prevention today is chemoprevention. A large and increasing number of patients in the world use medicinal plant and herbs for health purposes [17]. Brine shrimp lethality assay is an index of bi-directional biological activity, which suggests the bioactivity of the plant extract as well as the cytotoxic activity of the plant material tested [18]. Cytotoxic drugs have the potential to kill the cancerous cells and are usually developed after initial screening of thousands of lead compounds from various sources.

In the present study, the cytotoxicity study using Brine Shrimp Lethality Assay toxicity testing of *C. coromandelicum* fruit chloroform extract has shown that the plant is toxic. The chloroform extract have highest activity and having more bio active compounds. *Artemia salina* nauplii is one of the alternatives for the biological toxicity assays of herbal extracts and this test turned out to be significantly correlated with several other animal models. The preliminary toxicity data obtained by conducting the Brine Shrimp Lethality Assay gives LC_{50} values which are a convenient platform for further toxicity studies. The order of toxicity in fruit was chloroform >methanol >petroleum ether extracts. The findings on the active cytotoxicity of *C. coromandelicum* fruit extracts may support the therapeutic use of the plant as an alternative medicine.

CONCLUSION: The present study, showed the presence of various bio active metabolites such as flavonoids, steroids, tannins, saponin glycoside, cardio glycoside, sterol in different extracts of *Canthium coromandelicum* fruit that confirms, this is a potent source for modern drugs. All the pharmacognostical characters and physico-chemical parameters have been reported for the first time. Petroleum ether and chloroform extracts showed highest activity against *Entero coccus* and the methanol extract exhibit more activity against *E. coli.* The crude extract demonstrating significant antimicrobial activity could result in the discovery of novel antibiotics. The Brine Shrimp test represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and antitumor properties.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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